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LABORATORY PROCEDURE FOR EVALUATING THE CURD-PRODUCING CAPACITY OF SOYA PRODUCTS [1]

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ABSTRACT

A procedure for the rating of soya products as sources of soy curd, employing the essential steps of Asiatic soy curd processing, involves the aqueous extraction of the soy protein and its precipitation as a curd on the addition of magnesium chloride, the curd being measured volumetrically.

Appreciably lower curd yields are obtained from material larger than that which passes through a No. 100 U. S. Standard wire sieve. Maximal solubility and precipitation of protein is obtained when the extraction is conducted at a temperature of 80°C. for a period of thirty minutes with the suspension stirred mechanically. Salts of strong acids such as calcium chloride, magnesium chloride, ferric chloride, and sodium bisulphate are effective agents for the precipitation of soy proteins. Hydrochloric acid is also an effective curding reagent. Maximal yields of soy curd using magnesium chloride as the precipitating reagent occur at pH 5.8, whereas isoelectric precipitation using hydrochloric acid is at pH 4.5. Excessive quantities of the salts used to precipitate the soy proteins have given, within the concentrations studied, smaller yields of soy curd, the decreased yields being a characteristic of the precipitating salt not necessarily correlated with the pH of the final medium.

Data are presented showing that curd volume is an accurate index to the percentage of soluble protein in the soy flours.

The use of soya in relief feeding in both Europe and the Orient created new problems in description and control of products manufactured for different purposes. In Europe soya is used as a wheat flour extender, as a meat extender, and as a major ingredient for low cost food preparations; in the Orient it is used for the manufacture of soy sauce derived from the hydrolysis of protein, for "tofu" or soy curd prepared by the precipitation of water-extractable protein, and for "miso" or soy paste.

The present specification for soya products, Joint Army-Navy Specification JAN-S-588, refers to the protein as simply $N \times 6.25$, no consideration being given to the functional or nutritional properties of the foodstuff. A product may be satisfactory for one purpose, but

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fail in another. The evaluation of soya products utilized in the preparation of curd presented one of the greatest problems. This study concerns the development of a precise and rapid laboratory procedure for this purpose.

Materials and Methods

Samples of soy flour, representative of raw and heat-treated commercial products, were kindly furnished by members of the Soy Flour Association. In addition, Dr. James W. Hayward of Archer-Daniels-Midland Company, Minneapolis, Minnesota, in behalf of the Soy Flour Association, supplied data obtained in their analyses of the products according to conventional procedures.

The products consisted of (1) dehulled soy beans, (2) unheated solvent-extracted soy flour, (3) bakery type soy flour, mildly heat-processed in order to eliminate the bitter qualities of the raw bean, (4) soy flour optimally heat-processed with respect to the nutritive quality of the protein, and (5) an overheated processed flour experimentally processed for this study. These raw materials for flours were supplied in flake form. In addition, an expeller series was supplied, consisting of (6) dehulled beans which had been heat-treated, (7) expeller flour with some additional heating, (8) flour, heat-processed so the protein had optimum biological value, and (9) overheated soy flour. These samples were supplied in chip form.

Samples were found to be most easily ground with a W. J. Fitzpatrick Comminuting mill. Solvent extracted flakes were ground through a 1 mm. mesh sieve and then reground through a No. 40 wire sieve. Samples 1, 6, 7, 8, and 9, because of their appreciable fat content, were ground through a 1 mm. mesh sieve and then extracted with Skelly Solve "B" in a Soxhlet extractor for 16 hours. After thorough air-drying these were reground through a No. 40 wire sieve.

Optimum sifting time for all samples through a No. 100 U. S. Standard sieve was determined by the method of Wichser, Shellenberger, and Pence (7). All samples were sifted through a No. 100 wire sieve.

In the many soy curd methods used by the Orientals, the essential steps are (1) aqueous extraction of soluble protein, (2) separation of solution from residue, and (3) precipitation of protein by means of salts, mainly those of magnesium. The manner in which these steps are performed are many and varied but the results are comparable. When the whole bean is employed a soaking step may be utilized to remove the hulls and facilitate wet grinding. Time and temperature for extraction of the crushed or milled bean may vary widely from a few

minutes in boiling water to several hours in hot water. Separation of solution from residue is accomplished by decantation or filtration through a cloth. Precipitation methods employed by the Orientals differ as to the reagent used; however, salts of calcium and magnesium, i.e. gypsum, magnesium sulphate, calcium chloride, magnesium chloride, and mixtures derived from evaporation of sea water, are used almost exclusively. To establish reproducible conditions for the above steps the following basic procedure was used:

The extract was prepared by suspending 8.00 g. of soy flour in 100 ml. of distilled water, or multiple of this flour-water ratio, and stirred with a mechanical stirring device. After extraction the suspension was centrifuged in an International centrifuge No. 1 with trunnion cups, radius measurement 20 cm. to the tip, 1,800 r.p.m. for ten minutes. Twenty-five ml. of the extract liquid were pipetted into a 50 ml. long-tapered centrifuge tube graduated in fractions of ml. to 20 ml.; curding solution was added and the suspension diluted to the 50 ml. mark. Centrifuging was again accomplished at 1,800 r.p.m. for ten minutes.

Results and Discussion

Influence of Temperature on the Extraction of the Soy Proteins. Sample No. 3, bakery type soy flour, was selected for this study. Of the samples submitted, it contained an intermediate quantity of

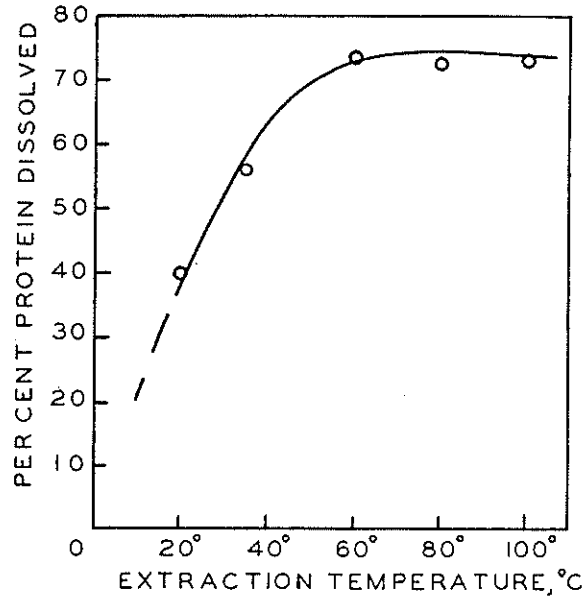

Fig. 1. The relationship of soluble protein and temperature of extraction; time, 60 min.; test material, bakery type soy flour (Sample No. 3).

soluble protein. The flour was extracted at several temperature levels for one hour. Protein analyses ($N \times 6.25$) of 25 ml. aliquots of the extract, after centrifugation, indicate maximum solubility of the protein at 60°C. to 100°C. as shown in Fig. 1. In another experiment designed to determine the effect of time, extractions were repeated at 80°C. for intervals of 10, 20, 30, 40, and 60 minutes. Fig. 2 shows that at 20 minutes, extraction has reached a maximum with no significant change on continued heating. Since 30 minutes and 80°C. extraction conditions are sufficient to insure maximum protein dispersion, a longer extraction period is unnecessary and undesirable because of

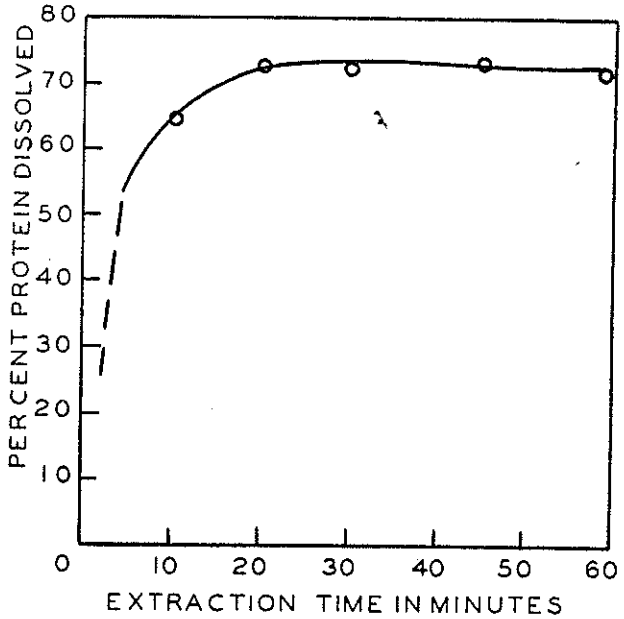

Fig. 2. The relationship of soluble protein, extracted at 80°C., to time; test material, bakery type soy flour (Sample No. 3).

water evaporation; this can lead to an erroneously high estimate of soluble protein.

Although the yield of soluble protein by this method of hot extraction was greater than that reported by the manufacturer for this sample,[4] viz. 70% as compared to 65%, it did not exceed the manufacturer's reported value for the raw sample (No. 2). This increased yield of soluble protein was evident in all samples and particularly in the heat treated soya products.

Optimum Concentrations of Curding or Precipitating Reagents. Several salts and hydrochloric acid were evaluated as precipitating

[4] Indirect Method; Archer-Daniels-Midland Company.

agents in this study. Sample No. 2, unheated soy flour, which exhibited greatest solubility, was used for the tests. Protein from 25 ml. of extract was precipitated with solutions of magnesium chloride, ferric chloride, sodium bisulphate, and hydrochloric acid. The final normality after dilution to 50 ml. was calculated. By subtracting twice the protein content of 25 ml. of supernatant solution after precipitation and centrifugation of the curd from the protein content of the extract, the weight of protein in the curd can be determined.

Fig. 3 shows that maximal yield of precipitated protein was attained when the solutions were 0.01 N to 0.03 N with respect to the salt and

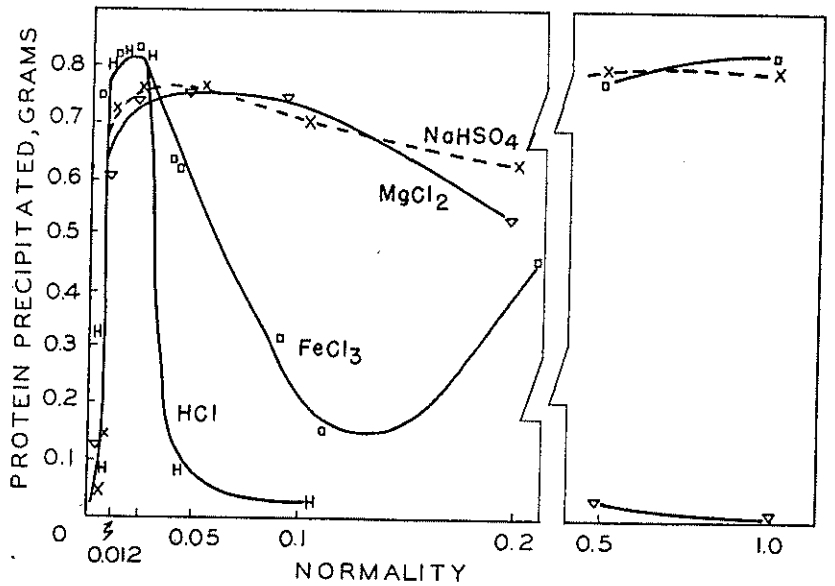

Fig. 3. Relation of salt and acid concentration to the yield of precipitable protein of soya; test material, defatted raw soya (Sample No. 2).

acid. This is in close agreement with the results of Smith *et al.* (6) who report minimum extraction of soybean nitrogen with calcium chloride solution of 0.0175 N and magnesium chloride solution of 0.025 N. Minimum dispersion of nitrogenous matter in acid was obtained at pH 4.2 by these authors (5). Maximum protein precipitated by hydrochloric acid was found in this study to be in the pH range of 5.0 to 4.0. Hydrochloric acid or isoelectric precipitation was noted to be slightly more efficient than magnesium chloride, yielding approximately 4% more of the available protein. The curves plotted in Fig. 4 indicate that the yield of curd is not necessarily correlated with the pH of the final medium.

The Effect of Heating upon the Solubility of the Protein and upon the Precipitation of the Curd. The data in Table I illustrate the effect of cold extraction and of hot extraction followed by curding from cold and hot solutions. Extraction at room temperature is not efficient under the conditions described. The curd volume was less than anticipated compared to the amount of protein in solution. The condition of the curd was fluid in nature and the residual protein after curding was large. By slightly heating the extract after the first centrifugation a more efficient precipitation was obtained, denoted by an increase in curd volume and decrease in quantity of protein in the top liquid.

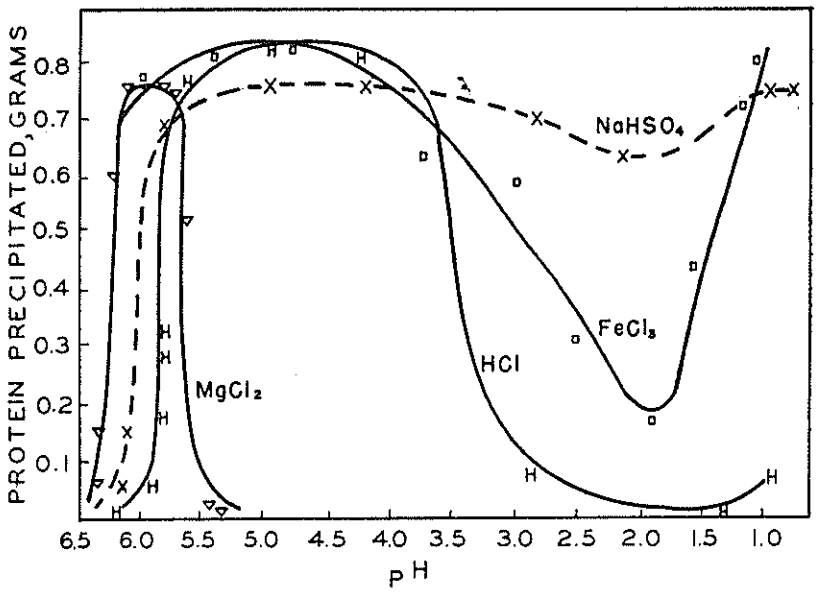

Fig. 4. The relationship of the yield of curd to the pH of three salt solutions and hydrochloric acid test material, defatted raw soya (Sample No. 2).

Thirty minutes extraction at 80°C. increases the yield of soluble protein (as was shown in Fig. 1), but cooling the extract at room temperature before protein precipitation gives a curd having undesirable small creamy particles which do not pack well, resulting in an apparently greater volume. Heating serves a two-fold purpose: (1) it produces the maximum yield of soluble protein in the minimum of time, and (2) causes incipient denaturation of the extractable protein, making it more readily precipitable following the addition of the curding reagent. The heat treatment yields a curd possessing good consistency and which is easily centrifuged, thereby improving the precision of the gravimetric separation.

The Effect of Granulation or Particle Size of Flour on Extraction of Protein and on Soy Curd Yield. Marked changes in curd yield by volumetric measurement were noticed with the same soy flour of different granulation. Samples of the unheated (Sample No. 2) and the optimally-heated (Sample No. 4) soy flours were used to determine the effect of granulation or particle size on the reproducibility of the

TABLE I

EFFECT OF HEATING UPON THE SOLUBILITY OF THE PROTEIN AND UPON THE PRECIPITATION OF THE CURD [1]

Extraction and curding method	Curding agent	pH curd suspension	Curd vol.	Distribution of protein[2]		
				Extract	Curd	Supernatant
			ml.	%	%	%
Two hrs. extract at 23°C.	$MgCl_2$	5.65	1.8		41.3	20.3
		5.65	1.9		40.1	21.6
Curding at 23°C.				61.6		
	HCl	4.80	1.9		47.6	14.0
		4.80	1.9		47.9	13.8
Two hrs. extract at 23°C.	$MgCl_2$	5.70	3.5		53.3	10.9
		5.70	4.0		53.3	10.9
Brought to 80°C. before curding				64.2		
	HCl	4.80	3.5		56.3	7.9
		4.80	3.5		56.1	8.1
Two hrs. extract at 23°C.	$MgCl_2$	5.68	5.5		53.8	10.2
		5.65	5.5		53.8	10.2
Protein solution heated 30 min. at 80°C.				64.0		
	HCl	4.60	5.5		56.7	7.3
		4.60	5.5		56.7	7.3
Thirty min. extract at 80°C.	$MgCl_2$	5.70	7.5		74.3	12.3
		5.70	7.5		74.3	12.3
Protein solution cooled to 23°C. for curding				86.6		
	HCl	4.60	7.0		78.2	8.4
		4.60	7.0		78.7	7.9

[1] Defatted raw soy flour (Sample No. 2) used in this study, 8 g. flour to 100 ml. water.
[2] Per cent protein calculated as per cent of total protein. Supernatant protein determined by Kjeldahl method on an aliquot of top liquid after precipitation. Protein of curd calculated by difference; extract protein minus protein of supernatant liquid after precipitation.

test. The Joint Army-Navy Specification for Soybean Products, dated May 10, 1948 specifies:

> "E-7. Ninety-seven per cent of the soy flour shall pass through a U. S. Standard 100-mesh screen."

The materials through and over a 100-mesh screen of Samples No. 2 and No. 4 were tested for soluble protein and curd yield. The results in Table II, average of duplicate tests, emphasize that large differences in per cent soluble protein and in curd volume are obtainable if the

Plotting curd volume against per cent soluble protein and per cent of protein precipitated, Fig. 7, illustrates the relationship of soluble protein to the yield of curd. Soy flours of low protein solubility have a residual protein after precipitation of from 8 to 10%. High protein solubility flours have a residual protein of from 10 to 14%, indicated by the dotted lines.

The data from Table II were plotted on the figure, represented by the letters Z and Y. Letter Z represents material through a 100-mesh

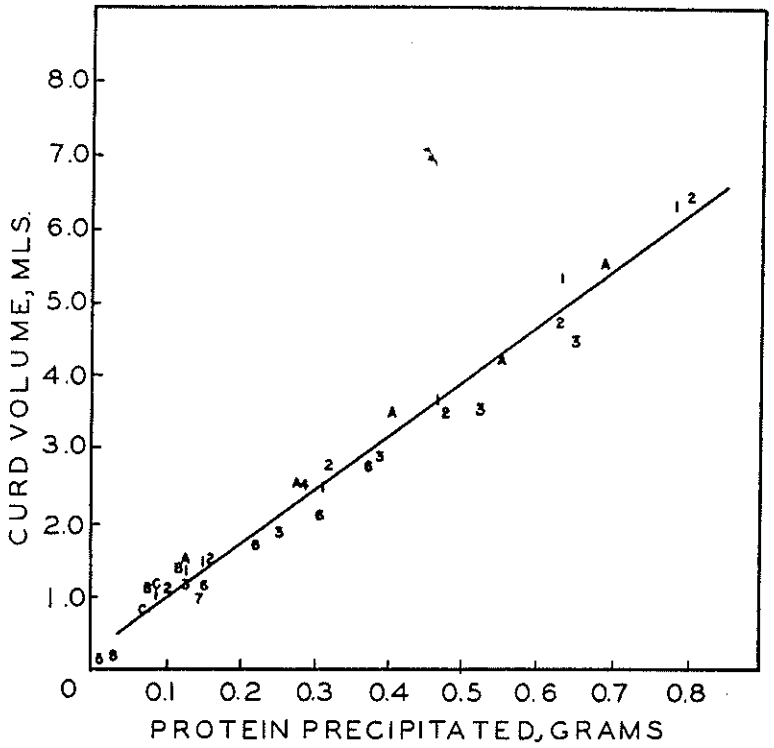

Fig. 6. Relation of protein precipitated to curd volume with thirty-five aliquots of eleven samples of soy flour and soy meal. The numbers refer to the soy flour test samples; the letters refer to solvent extracted soy meals.

sieve and Y the material over the wire from Samples No. 2 and No. 4. It is again evident that the yield of curd is dependent upon the amount of extractable protein.

A curd volume of not less than 4.0 ml. by this method for solvent-extracted soy flours and meals would identify such products as the type which would find acceptance with Orientals. Alternately, heat-treated products similar to samples 4 and 5 would be useful as far as

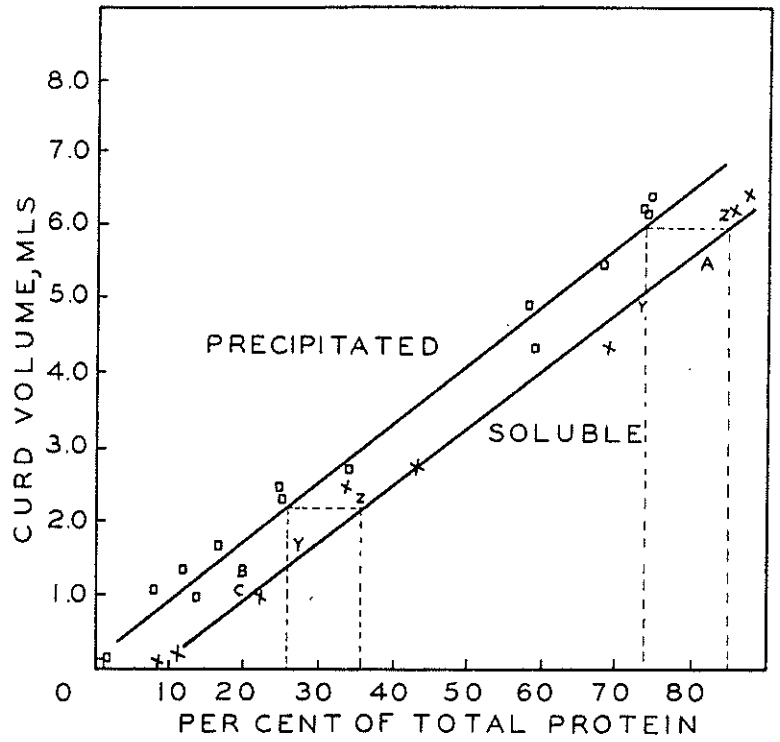

Fig. 7. Relation of per cent soluble protein in soy products and per cent of protein precipitated to curd volume.

consumption *in toto* is concerned, in soup mixes, and as protein supplements in cereals, but would be unsatisfactory for curd formation.

These studies have established that a good correlation exists between soy curd volume, as determined by this method, and quantity of protein precipitated, justifying the use of the far simpler volumetric procedure rather than the nitrogen analysis for the determination of the relative concentrations of the precipitable soy protein.

The method recommended is as follows:

Sample preparation:

Sample should be of such granulation that it passes through a No. 100-mesh U. S. Standard screen.

Apparatus:

(1) Centrifuge bottles, Pyrex, 250 ml.
(2) Pipettes, 25 ml.
(3) Waterbath.
(4) Mechanical or electrical stirring device with glass stirring rod.

(5) Centrifuge, International No. 1 or equivalent, radius 8 in. (20 cm.) to tip, trunnion mount cups.
(6) Centrifuge tubes, long taper, 50 ml., graduated in fractions of ml. to 20 ml.
(7) Magnesium chloride solution, 0.20 *N* to 0.25 *N*.

Determination:

Weigh 8.00 g. soy product (8.0% moisture basis) and place in centrifuge bottle, add 100 ml. of distilled water and stir with glass rod from stirring device until all the material is in suspension and none adheres to the sides.

Place in water bath, 80°C. ± 2°C., and start stirring device. Extraction is continued 30 min. after temperature of the solution

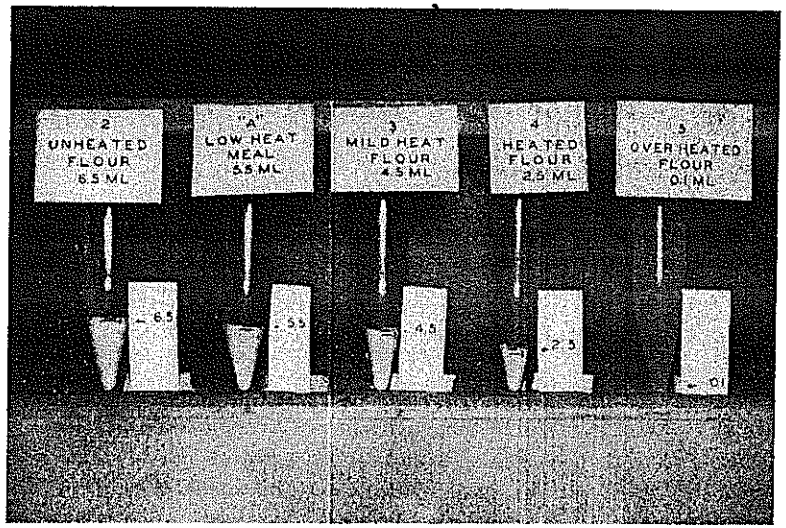

Fig. 8. Illustration of five samples of defatted soya at the completion of the curd test.

passes 60°C. (approximately 5 min.). Remove bottle and centrifuge at 1,800 r.p.m. for 10 min.

Pipette 25 ml. of the supernatant liquid into a long-tapered centrifuge tube, bring solution to 80°C., remove tubes and add 5 ml. of 0.2 *N* magnesium chloride solution while agitating the tube. Dilute to the 50 ml. mark with water and centrifuge at 1,800 r.p.m. for 10 min. Allow centrifuge to come to rest without braking and read curd volume to the nearest fraction of a ml. Two to three tests may be conducted on the extract for increased precision.

Fig. 8 illustrates the appearance of the centrifuge tubes at the completion of the curd test on five soybean products. The reproducibility of the soy curd test, the ease of running such assays, and its direct applicability to evaluation of soya products intended for use in the Orient, justify inclusion of the method in an amendment to the present Joint Army-Navy Specification for the identification of soya products. In current investigations conducted in the Institute by Simon and Melnick (4), it has been suggested that the soy curd test has further value in defining heat-processed soya products with respect to the nutritive value of the protein.

It was previously mentioned that magnesium chloride precipitation of the soy protein occurs at a pH somewhat removed from the isoelectric point. Precipitation with hydrochloric acid yields a soy curd comparable to that formed following the use of magnesium chloride as the curding reagent but differs slightly in the yield of precipitated protein. In order to determine if the difference in curd yield was due to some protein fraction which was precipitated by hydrochloric acid at the isoelectric point and not precipitated by magnesium chloride, electrophoretic analysis was employed. A water extract of Sample No. 2 unheated soy flour was prepared according to the test method and divided into two portions. Magnesium chloride reagent was added to one portion and hydrochloric acid to the other in concentrations which produced maximum yield of curd. Electrophoretic analyses of the protein in the two supernatant solutions, kindly conducted by Dr. A. C. Shuman, General Foods Corporation, Hoboken, New Jersey, indicated that the two solutions were identical as far as their electrophoretic mobilities were concerned and each consisted of a single component with a small degree of non-homogeneity. Thus, precipitation at the isoelectric point yields the same protein precipitate as the procedures customarily employed by the Orientals.

In this connection, it is of value to speculate on the possible fate of the antitryptic factor in the raw or mildly-heated soya products which are of the type most favorable for soy curd formation. Such mildly-heated products contain the antitryptic factor, which has been held to be responsible for poor protein-utilization *in vivo* (2, 3). Precipitation with hydrochloric acid at the isoelectric point has been employed (1, 3) to remove irrelevant protein leaving in solution the antitryptic complex. It seems quite likely from the electrophoretic studies that the magnesium chloride reagent would also be effective for this purpose. However, the efficiency with which the antitryptic complex can be isolated by these procedures has not been adequately studied. The possibility of co-precipitation of the antitryptic complex with the soy curd does exist. This point of importance in predicting the value of the soy curd as a source of protein of high biological value is now under investigation in our laboratories.

Acknowledgments

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Literature Cited

1. BORCHERS, R., ACKERSON, C. W., MUSSEHL, F. E., and MOEHL, A. Trypsin inhibitor, VIII. Growth inhibiting properties of a soybean trypsin inhibitor. Arch. Bioch. 19: 317–322 (1948).
2. HAM, W. E., SANDSTEDT, R. M., and MUSSEHL, F. E. The proteolytic inhibiting substance in the extract from unheated soybean meal and its effect upon growth in chicks. J. Biol. Chem. 161: 635–642 (1945).
3. KLOSE, A. A., HILL, B., and FEVOLD, H. L. Presence of a growth inhibiting substance in raw soybeans. Proc. Soc. Exp. Biol. Med. 62: 10–12 (1946).
4. SIMON, M., and MELNICK, D. The *in vitro* digestibility of raw and heat-processed soy products varying in the nutritive value of the protein. Cereal Chem. 27: 114–126 (1950).
5. SMITH, ALLEN K., CIRCLE, S. J., and BROTHER, G. H. Peptization of soybean proteins. The effect of neutral salts on the quantity of nitrogenous constituents extracted from oil-free meal. J. Am. Chem. Soc. 60: 1316–1320 (1938).
6. SMITH, ALLEN K., and CIRCLE, S. J. Peptization of soybean proteins. Extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. Ind. Eng. Chem. 30: 1414–1418 (1938).
7. WICHSER, F. W., SHELLENBERGER, J. A., and PENCE, R. O. Relationship of physical properties of wheat flour to granulation. Cereal Chem. 24: 381–393 (1947).